REMARKS

Status of the claims

With entry of this amendment, claims 15 and 30 have been amended and claims 20 and 33 have been cancelled. Claims 15, 17, 22-30, 32, and 34-42 are therefore pending and under examination.

Cancellation of subject matter is without prejudice to subsequent revival for prosecution in a continuation filing.

The amendments add no new matter The elements set forth in claims 20 and 33 have now been incorporated into claims 20 and 33.

The claims are fully enabled.

The claims are rejected under 35 U.S.C. § 112, first paragraph as allegedly not enabled. In the rejection, the Examiner contends that it would take undue experimentation to identify members of the genus of sequence non-specific double-stranded nucleic acid binding proteins that have at least 75% or at least 85% identity to Sso7d or Sac7d; or at least 90% identity to Sac7d; and that can enhance processivity of a polymerase. In the current final Office Action, the Examiner has now cited three publications that allegedly further support the rejection. These will be addressed in the "*References*" section below.

Applicants have traversed the rejection for reasons of record, which will not be reiterated at length in this response. However, it is noted that claims 15 and 33 are now amended to recite at least 85% identity to a reference sequence. Thus, these claims relate to a modified polymerase that has a sequence-non-specific double-stranded nucleic acid binding domain that comprises an amino acid sequence that has at least 85% identity to SEQ ID NO:2 (Sso7d) or to the Sac7d sequence of SEQ ID NO:10. The examples in the specification show that both Sac7d and Sso7d work in the claimed invention. These two proteins, relative to one another, are two of the most divergent members of the naturally occurring family members (*see, e.g.,* section 7 of the Vander Horn Declaration previously of record in this application). Sso7 protein variants that have at least 85% identity to Sso7d (or Sac7d) are more closely related to the reference sequence than are Sac7d and Sso7d to each other. For example, in reviewing the alignments provided in

Dr. Vander Horn's Declaration, with regard to Sso7d, there are 12 residue positions of the 63 residues in which natural variation is known. The limit of 85% identity would encompass variants that have less than this full range of variation, but still allow most changes that could be introduced into an Sso7d sequence based on the naturally occurring variation. For the reasons explained in the Vander Horn Declaration, such changes would reasonably be expected to retain function, as the naturally occurring family members have the same function. The same reasoning applies to proteins having at least 85% identity to Sac7d. Accordingly, claims drawn to protein domains having at least 85% identity to Sso7d or Sac7d are additionally enabled.

Cited references

In the Office Action, the newly cited references are characterized as showing that a single point mutation in Ssod can affect the function of the nucleic acid binding domain. However, the references in fact support the enablement of the claims. Submitted herewith is a Declaration under 37 C.F.R. § 1.132 by Dr. Yan Wang, which explains that the experiments performed in the cited publications provide evidence that one of skill can successfully employ the extensive structural Sso7d/Sac7d data available in the art to predict the effects of sequence changes on Sso7d (or Sac7d) function. In the cited references the authors were seeking to investigate Sso7d by introducing mutations that were predicted, based on the structure, to negatively affect function. Dr. Wang illustrates how their results validated this approach. In the current invention, the skilled artisan can use this same structural information to reasonably predict sequence changes that preserve Sso7 function rather than destroy it. Each of the references is individually discussed below.

Wang, *et al. Nucl. Acids Res.* 32:1197-1207, 2004 ("Wang")

The Examiner points to Wang as further supporting the rejection because Wang teaches that a change in Trp24 of Sso7d significantly reduces the effectiveness of the protein in enhancing processivity. Wang is a post-filing publication of the current inventor's work relating to polymerases that are modified by linkage to an Sso7d protein. Dr. Wang explains that in one aspect of the experiments presented in the article, it was determined that Sso7d double-stranded

DNA (dsDNA) binding activity is important for processivity, as taught in the current application. As Applicant has previously noted, the interactions between Sso7d and dsDNA have been extensively studied. Dr. Wang explains that for the experiments described in the Wang reference, Trp 24 was identified in structural studies to be important for binding to dsDNA, as explained on page 1201, column 1 in the last paragraph. (Trp24 in Wang corresponds to Trp23 in SEQ ID NO:2 of the application as filed.) The referenced structural studies (Gao *et al., Nature Struct. Biol.* 5:782-786, 1998; and Catanzano, *et al. Biochemistry* 37:10493-10498, 1998) were readily available in the art before the current invention. Dr. Wang purposefully selected Trp 24 for mutation to further investigate the correlation between DNA binding and processivity. Three mutant Sso7d-polymerase fusion proteins in which Trp 24 was replaced with Val, Gly or Glu were created with the intent of reducing the ability of Sso7d to bind dsDNA and in turn, reducing its ability to enhance the processivity of the DNA polymerase. Dr. Wang points out that all three mutant fusion proteins exhibited decreased processivity relative to that of the wildtype Sso7d-polymerase fusion (see, the first column of page 1201 bridging to the second column), just as they had expected. Substitution of Trp 24 with Glu, which had been expected to exhibit the greatest effect because it differs the most from the wild-type residue, also resulted in the greatest decrease in processivity.

Dr. Wang also points to Tale 2 on page 1202, noting that all three mutant Sso7d proteins still retained at least some ability to enhance processivity when compared to the unmodified polymerase. Thus even changing this important residue did not completely destroy Sso7d function in enhancing processivity.

The experiments presented in Wang therefore illustrate how one of skill in the art makes use of structural information to recognize amino acid residues that are expected to be relevant to function. Wang and co-authors intentionally selected a residue based on available Sso7d structural data, fully expecting to compromise the function of Sso7d in enhancing polymerase processivity. This is precisely what was observed. The same structural information can be used to select residues that would <u>not</u> be expected to alter Sso7d activity, as Applicant has explained in previous responses to this rejection that are of record in this application.

Consonni *et al., Biochemistry* 38:12709-12717, 1999 ("Consonni")

Consonni is cited by the Examiner as providing evidence that the claims are not enabled because a single amino acid change (at Trp 23 or Phe 31) in Sso7d can alter function. However, Consonni also provides another example of how structural information is predictive of the functional importance of particular amino acid residues. Dr. Wang explains that Consonni describes the solution structure of an Sso7d mutant protein F31A, in which an alanine is substituted for a phenylalanine residue at position 31. In prior studies cited in Consonni at page 12710 in the second full paragraph of the first column, Phe 31 was selected for mutation on the basis of structural data that indicated that this residue is located at the core of the aromatic cluster and has tight contact with side chains of several residues in the cluster. This residue was therefore predicted to be important for stability.

Dr. Wang further notes that this residue is also highly conserved in Sso7 family members, as can be seen in a sequence comparison of Sso7d, Sac7d, Sac7a, and Sac7e (see, the Rule 1.132 Declaration by Peter Vander Horn of record in this application) As the authors expected, the mutation of Phe 31 to Ala led to a loss in thermo and piezostabilities (third paragraph of column 1, page 12710). The analysis presented in the current Consonni paper relates to the solution structure of the F31A mutation, which was performed in order to determine the structural changes that were associated with the loss of stability of the mutant protein.

Dr. Wang points out that Consonni observed that in the solution structure of the F31A mutant, the Trp 23 residue was reoriented such that it pointed inside the aromatic cluster. Because of the previously identified role of Trp23 in contacting DNA (Trp 23 is the same residue as Trp24 in Wang), Consonni investigated the DNA-binding activity of the mutant F31A protein. The results showed that the binding activity was also impaired, once more highlighting that Trp 23 plays an important role in DNA binding, as indicated by the structure. Consonni thus provides a further example of how a practitioner in this art makes use of structural information.

With regard to the loss of stability observed in the F31A mutant protein, Dr. Wang indicates that it is not surprising that the mutation affected Sso7d stability. As explained in the Declaration, it is well known in the field that an amino acid with a large, buried hydrophobic side chain stabilizes conformation. Accordingly, it is predictable that changing the large hydrophobic

side chain to a small side chain would result in a loss of stability. In designing mutations that are expected to preserve function, Dr. Wang further notes that it is standard practice in the art to avoid radically mutating such residues, if it is desired to preserve function, just as it would be desirable to avoid mutating those residues that directly contact DNA to preserve DNA binding function.

Shehi, *et al. Biochemistry* 42:8362-8368, 2003 ("Shehi")

Shehi performed studies examining the thermal stability and DNA binding activity of Sso7d. Dr. Wang reviews the results of these analyses and then addresses the specific issues raised by the Examiner.

Shehi structural analysis

Shehi investigated the function of the C-terminus of Sso7d. Shehi created an Sso7d protein that was truncated at Leu54 (L54Δ) in order to investigate the role of the C-terminal α-helix on stability and DNA binding activity. Dr. Wang notes that this region does not contact the DNA in the structural analysis of Sso7d and Sac7d DNA binding interactions. Dr. Wang then explains that to determine whether deletion of the C-terminal region had effects on DNA binding, the authors analyzed the binding of L54Δ to double-stranded calf thymus DNA in comparison to the binding activity of wildtype Sso7d. Dr. Wang points out that the association constant for binding of L54Δ to double stranded DNA was similar to that of Sso7d (page 8362 bridging to page 8363 and Figure 4), thus showing that deletion of the eight residues at the C-terminus of Sso7d did not result in loss of DNA binding activity, which was predictable based on the structure.

The authors also observed that a variant that was truncated at Glu 53 could not be isolated under the same conditions that allowed them to isolate L54Δ and noted that this highlights the role that Leu 54 plays in the folding process. Shehi observes that Baumann and colleagues (*Nat. Struc. Biol.* 1:808--809, 1994)) in fact described that the side chain of Leu54 is packed well against that of Ala50, anchoring the C-terminal end of the chain to the protein core. Other investigators also confirmed that Leu54 is involved in strong van der Waals interactions with the remaining part of the protein. Thus, as Dr. Wang explains, the available Sso7d/Sac7d

structural data provided information on the role of Leu 54 that was born out by the studies in Shehi.

Dr. Wang further describes that Shehi's results are consistent with the analysis of Sso7d structure provided by Dr. Vander Horn in his Declaration that is of record in this application. Dr. Wang points out that Dr. Vander Horn has indicated that in the context of DNA binding activity, the alpha helix is highly mutable, as evidenced by the fact that natural variation of Sso7 homologs is observed in this domain. Dr. Vander Horn cautioned, however, that the naturally occurring mutations in this domain appear to preserve the alpha helix. Thus, in designing Sso7d variants for use in the invention, one of skill would introduce mutations that preserved structure. Dr. Wang further notes that the L54 residue is also conserved across the naturally occurring Sso7 proteins, which also would be an additional consideration in designing variants with the purpose of retaining DNA binding activity.

Examiner's rejection

Shehi mentions that there were difficulties in isolating the deletion in which the C-terminus was truncated at Glu53 under the same conditions that were used to isolate L54Δ. Shehi also noted that L54Δ has a limited solubility in aqueous solution. The Examiner contends that "both mutations demonstrate the unpredictability of the effect of point mutations in Sso7d on any particular function or attribute of Sso7d." However, Dr. Wang explains that one of skill cannot conclude from the experiments in Shehi that the effects of point mutations at Glu53 or L54 would be unpredictable. Dr. Wang first notes that Shehi investigated deletion mutations, not point mutations, and that the effects observed in deleting most of the C-terminal α-helix cannot be extrapolated to the effects of introducing point mutations into that region.

In terms of the limited solubility of L54Δ, the authors believe that this is likely due to the loss of three net charges and the exposure of hydrophobic moieties upon deleting the last eight residues. Dr. Wang points out that it is recognized in the art that changing the charge of a protein and exposing hydrophobic residues can influence solubility. She indicates that a practitioner in this art can additionally consider such effects in designing variant Sso7d sequences. Last, she notes that Shehi was examining L54Δ alone, not when fused to a

polymerase protein. This is relevant because, the limited solubility observed by Shehi under these conditions would not necessarily reflect the solubility when the protein is fused to a polymerase. In view of the foregoing, the studies in Shehi do not provide evidence that the current claims are not properly enabled.

Summary

The standard for enablement is that one of skill be able to practice the invention with a reasonable expectation of success. It is well-settled in the biotechnology art that routine screening of even large numbers of samples is not undue experimentation when a probability of success exists. (*In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). As illustrated in the Declaration by Dr. Wang, the three references cited in the current final Office Action demonstrate that the structural information about Sso7d/Sac7d sequence and function provide a sound basis for accurate prediction of effects of Sso7d/Sac7d mutations on DNA binding function. In view of the foregoing and the arguments previously of record in this application, the claims are fully enabled.

CONCLUSIONS

Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Jean M. Lockyer
Reg. No. 44,879

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
JML:jml
61093387 v1